A STUDY OF THE HEARTS OF ALBINO MICE MAINTAINED ON A LOW PROTEIN DIET

A THESIS

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GRACE HEYWARD

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CHAPTER I

INTRODUCTION

Griffith and Wade ('39) studied the effects of low choline diets on male rats. It was observed that hemorrhagic kidneys, fatty livers, enlarged spleens and a decrease in the size of the thymus gland resulted when animals were fed on a high fat low protein content diet. Patterson and McHenry ('42) studied the effects of choline on albino rats and reported that the administration of lipotropic supplements such as choline and triethylcholine completely prevented the appearance of kidney lesions. However, when these lipotropic agents were not received, decrease in weight and a decrease in phospholipid percentage concentration were observed. Tucker and Eckstein ('37) studied the effects of supplementary methionine and cystine on the production of fatty livers in male rats. It was observed that these sulfur-containing amino acids exerted opposite effects on the production of fatty livers. Wilgram, Best and Blumenstein ('55) studied the aggravating effects of cholesterol on cardiovascular changes in choline deficient rats and observed that cardiovascular lesions occurred when an addition of cholesterol was added to a choline depleted diet.

In light of these findings, a study was made in an effort to learn what affects a low protein diet fed over a period of 10 days would have on the heart of mice.

CHAPTER II

REVIEW OF LITERATURE

Patterson and McHenry ('42) studied the effects of choline on 95 young male albino rats of the Wistar strain for 10 days. The initial weights of these animals were between 40 and 45 gms. They were housed in individual screened containers and water and food were supplied ad libitum. The effects of 5 types of diets were studied. The low basal choline diet, Diet I, contained the following percentages of ingredients: casein 10, corn oil 30, salt mixture 4, sucrose 53.5, agar two, and l-cystine five-tenths. To every gram of diet, five-hundredths gram of cod liver oil concentrate was added to supply vitamins A and D. Each animal received a daily supplement, given by injection, of 20γ thiamine chloride, 20γ riboflavin, 100γ calcium pantothenate, and 20γ pyridoxine. Diet II consisted of the low basal choline diet in addition to choline; Diet III the low basal choline diet in addition to triethylcholine (three milligrams per gram of food); Diet IV, the low basal choline diet plus 5 mgs. of triethylcholine per gram of food, and Diet V, the low basal choline diet plus two milligrams of choline per gram of food.

Five series of animals were used in this experiment. Series I to IV were used to determine the lipotropic effects of choline or triethylcholine on the diet. Each of these series contained 10 animals, 5 controls and 5 experimentals. Series I and II received Diets I and II, Series III received Diets I and III and Series IV received Diets I and IV. All animals were killed on the tenth day. The experiments on the animals which comprised Series V were designed to determine the rate of production of hemorrhagic kidneys and liver phospholipids. This series consisted of 55 rats, 5 of which were killed on

the first day. The remainder were divided into two groups, one of which received Diet I and the other Diet V. Five animals from each group were killed on the second, 4th, 6th, 8th and 10th day of the experimental period. By the end of the 10th experimental day the animals which received Diet I showed loss of body weight, paralysis of the hind limbs, loss of hair and a hunched position. The kidneys of most of these animals were enlarged and hemorrhagic. It was observed that the livers showed typical fatty infiltration and were slightly larger than those of the animals which were fed Diets II, III, or IV. A gradual increase in weight and in phospholipid percentage concentration were observed in animals which were fed Diet V.

The conclusion was reached that the administration of lipotropic supplements such as choline and triethylcholine completely prevented the kidney lesions. A gradual increase in weight and an increase in phospholipid percentage concentration were also observed which was in contrast to the changes in the kidneys of rats that did not receive a lipotropic agent.

Tucker and Eckstein ('37) studied the effect of supplementary methionine and cystine on the production of fatty livers in male rats. The basal diet, Diet I, contained the following percentages of ingredients: casein 5, glucose 48, agar two, lard 40, and salt mixture 5 (Osborne and Mendel '19). The effects of three diets were studied: basal diet, Diet I; the basal diet plus five-tenths per cent cystine, Diet II; and the basal diet plus five-tenths per cent methionine, Diet III. The experiment was set up with the following series:

Series I

Diet I was fed to 6 rats
Diet II was fed to 5 rats

Series II

Diet I was fed to 21 rats
Diet II was fed to 57 rats
Diet III was fed to 93 rats

Series III

Diet I was fed to 5 rats
Diet III was fed to 6 rats

The experimental period lasted between 18 and 19 days and the rats were killed by means of decapitation.

It was observed that when cystine was added to the diet low in casein and high in fat, the amount of fat in the liver was markedly increased. The average fat content of the livers of rats on the 5% casein diet was 26.8% as compared with 40.9% for the animals fed Diet II. The administration of Diet II resulted in an increase of 52% in the liver fat with a corresponding increase of 25% in the weight of the liver. In Series II the livers of rats that received Diet II contained more fat than those of rats fed the control diet, Diet I. All of the animals in Series II gained weight, however, the livers of rats which were fed Diet III contained less fat than those of either of the other groups in Series II.

The differences were significant enough to warrant the belief that, under the conditions of this experiment, the two sulfur-containing amino acids, when added to the diet, exerted opposite effects on the fat metabolism of the liver. The average value for the liver fat of rats fed Diet II was 33% as compared with 11% which was the average value obtained when they were fed Diet III. In those which received Diet I, the liver fat was approximately 20%. Results showed a 62% liver fat increase associated with Diet II as compared with a 46% decrease in liver fat associated with Diet II. On the other hand a 27% liver weight decrease was associated with Diet III with a

corresponding 24% liver weight increase associated with Diet II. In Series III the average fat content of the livers of 5 rats on Diet I was 18.9% as compared with that of 11.9% average value of fat content of the livers of 6 rats on Diet III.

Griffith and Wade ('39) studied some effects of low choline diets on male rats which were 24 days old at the beginning of the experiments. These animals weighed 40 gms. each and were used in groups of 10. The basal diet consisted of the following percentages of ingredients: fibrin-4, casein-8, dried egg white-three, salt mixture-4 (Hawk and Oser '31), calcium carbonate-one, cod liver oil-5, lard-35, agar-two, and sucrose-38. Water soluble vitamins were supplied by a daily supplement of two-hundredths milligrams of riboflavin, four-hundredths milligrams of nicotinic acid and one-tenths cubic centimeter each of concentrated extracts of rice polish and hog liver. The rats consumed 4 to 5 gms. of food per day. The term fatty liver referred in every case, to enlarged livers, which contained from 8 to 12 times the normal weight of chloroform-soluble substances. The experiment covered a period of 10 days. A gross and histological study was made of the tissues.

At the end of the 10-day experimental period 90% of the rats had marked hemorrhagic kidneys as well as fatty livers unless choline was added to the diet. Similar results were obtained when the vitamin supplement was omitted, fed separately, mixed with the basal ration or doubled in amount. The degeneration of the kidneys was prevented if the rats received two milligrams of added choline daily, however, this had no effect on the liver fat. Ten milligrams of choline per day were required to prevent the development of fatty liver. A high fat diet was not required for the production of the deficiency due to low dietary choline. The typical fatty livers and hemorrhagic kidneys occurred after having been fed on the basal diet in which the lard was

decreased from 35 to 15%. The deficiency condition was severe if all of the dietary protein was supplied by fibrin. The livers were fatty but the kidneys were normal if all of the protein consisted of either casein alone or of dried egg white alone.

Other indications of a severe pathological deficiency condition observed were noticeable sickness, enlarged spleen, and a uniform decrease in the size of the thymus to approximately one-half its normal weight. Microscopic examination of kidney tissue showed extensive glomerular and tubular degeneration with hemorrhagic areas in the cortical region particularly.

The importance of the absolute and relative amounts of certain amino-acids in determining the choline requirements was suggested by the differences in results obtained with casein or egg white, and with fibrin. Fatty livers occurred regardless of the protein used. Much larger amounts of choline were required to prevent the development of fatty livers than to prevent the renal lesions. This indicated that certain amino acids in casein and in the protein of egg white might have given small amounts of choline in the basal ration so that none of the deficiency symptoms appeared except the fatty liver when these proteins were used. The effect of small amounts of choline in preventing hemorrhagic degeneration of the kidneys demonstrated its important role in the maintenance of normal kidney structure in young rats.

Wilgram, Best and Blumenstein ('55) studied the "aggravating" effect of cholesterol on cardiovascular changes in choline deficient rats. The strain of animals used in this experiment was known to be rather resistant to the production of cardiovascular disease by choline deficiency. Seventy females which weighed 125 gms. and 21 males which weighed between 150 and 160 gms. were used in this experiment. The effects of 5 types of diets were studied.

The basal diet, Diet I, contained the following percentages of ingredients: casein 7, peanut oil 28, soya protein 5, salt mixture three, sucrose-vitamin mixture one, sucrose 10.15, lard 34, starch 6, cholesterol 4, choline chloride eighty five-hundredths, cinnamon three-hundredths, a-tocopheral acetate fifteen-thousandths, cod liver oil one-hundredths, corn oil one. Diet II consisted of the basal diet less choline chloride; Diet III, the basal diet with a two per cent decrease in the amount of cholesterol; Diet IV, the basal diet with a one per cent portion of cholesterol; and Diet V, the basal diet less choline chloride and cholesterol. Animals were sacrificed 10 weeks later to make sure no lesions were missed. Animals which received Diet IV showed an increase in the incidence of kidney and cardiovascular lesions slightly more than the animals which received Diet I. Animals which received Diet III showed a marked increase in the incidence of kidney and cardiovascular lesions. Practically all animals which died, succumbed to hemorrhagic kidney lesions. There appeared to be a close connection between the occurrence of hemorrhagic kidney lesions and cardiovascular diseases. Animals on Diet V showed cardiovascular changes to a lesser degree than did the ones fed Diet II. Animals fed Diet I thrived and grew well. Results indicated that increased amounts of cholesterol is much less harmful when choline is present in the diet. The animals fed Diet I were perfectly healthy, but the fat content of their livers was somewhat increased. Significant changes were not discerned in the cardiovascular system.

It was concluded that cholesterol supplements "aggravated" the occurrence of renal cortical necrosis and of cardiovascular lesions in rats on a high fat choline-deficient diet. Cholesterol supplements of 4% and two per cent were very effective in the production of cardiovascular lesions and some

change was seen in the animals which received a supplement of one per cent. The concomitant increase in kidney as well as cardiovascular lesions favored the hypothesis that the cardiovascular lesions induced by acute choline deficiency were linked to kidney damage as most animals with cardiovascular lesions died either from hemorrhagic kidney lesions, or showed at least some degree of renal frosting. A diseased kidney might conceivably be unable to regulate phosphorous and calcium levels in plasma and tissues and the resulting electrolyte disturbance might favor the production of vascular damage.

Rats have a greater ability to form phosphatidyl choline than the chick. This might help to explain why on one hand rats are rather resistant to cholesterol-induced arteriosclerosis and why, on the other hand, cholesterol, by increasing the demand for phosphatidyl choline, enhances the severity of the lack of choline in a choline-deficient animal.

CHAPTER III

MATERIALS AND METHODS

The animals used in this experimental research were 30 male albino mice which weighed between 12.2 and 19.5 gms. These animals were secured from Rockland Farms, New City, New York. They were divided into two groups; Group I was used as controls and Group II as experimentals. Group I was comprised of 10 animals and Group II of 20 animals.

The control animals were fed Walter Kendal "Hunt Club" homogenized dog meal which contained:

Crude protein	not less than 25%
Crude fat	not less than 7%
Crude fibers	not more than 4%
Ash	not more than 10%
Nitrogen free extract	not less than 45%
Moisture	not more than 10%
Vitamin A	3000 U. S. P. units per pound
Vitamin D_2	1000 U. S. P. units per pound
Vitamin B_{12}	.01 Mgms. per pound
Thiamine (B_1)	1.25 Mgms. per pound
Riboflavin (F_2)	2.00 Mgms. per pound
Niacin	16.00 Mgms. per pound
Pyridoxine (B_6)	1.00 Mgms. per pound
Choline chloride	700.00 Mgms. per pound
d-Pantothenic acid	4.00 Mgms. per pound
Phosphorus	not less than 2%

Calcium	not less than 1%
Iron	not less than .05%

The experimental animals were fed a wet filter-paper diet which was composed of:

Filter paper	4.7%
Casein	.8%
Wheat starch	7.0%
Peanut oil	3.5%
Salt mixture	4.0%
Cod liver oil	4.0%

In order to form a semi-liquid paste 76% water was added to the above items. Two vitamin B Complex tablets per 100 gms. of diet and three-fourths gram of wheat germ oil (a source of vitamin E) per 1000 gms. of diet were added to this semi-liquid paste.

The intake of food was regulated by placing each control in a battery jar. The experimental animals were placed in pairs and the data concerning them was averaged. The amount of food eaten by the control was then fed to the experimental the following day. Troughs were used with a small opening just large enough for the head of the mouse so as to prevent scattering of food. However, inspite of precautious measures, some food was dispersed. The food which was scattered was separated from the feces daily. The food which was recovered was added to the food in the trough and weighed.

Each day over the 10-day experimental period, one control and two experimentals were weighed and sacrificed by a blow on the head. The heart was dissected out and fixed in 10% formalin. Paraffin sections were prepared and stained with Harris' haematoxylin and Sudan IV (Scharlach R).

CHAPTER IV

EXPERIMENTAL RESULTS

Observations made early during the experimental period indicated that the mice lost weight and the normal smooth appearance of their coats. The coats seemed somewhat coarse and dull in texture. Throughout the entire experimental period the skin of the tail and feet remained unchanged. The feces, though well formed, became increasingly pale and the animals grew more sluggish as the experimental period progressed. There was no indication of secondary infections.

The changes with respect to the food intake and body weights of animals fed on the control and low protein diets may be found in Table 1. A complete loss of appetite did not occur. However, the mice which were fed the diet low in protein consumed on an average less food than did the controls and a steady loss of weight was observed. On autopsy, inspection revealed that no apparent changes were present in the color or the overall condition of the hearts.

Histological sections through the ventricular and blood vessel regions of the experimental and control hearts were studied. Sections from comparable regions of the experimental hearts were no different from those of the control. Photomicrographs of sections from the controls are shown in figures 1-3, and from the experimentals in figures 4-10.

CHAPTER V

DISCUSSION

Experimental results indicate that animals fed on the control as well as the experimental diets received rations that were sufficient for the maintenance of a normal heart. Results also revealed that, whereas the ration of the experimental diet was sufficient to maintain a normal heart, there was a loss of weight which is in agreement with the findings of Patterson and McHenry ('55).

Hartroft and Ridout ('51) produced cirrhosis in the livers of 250 rats fed a low protein diet for an experimental period of 6 months. These investigators concluded that ruptured fatty cysts might have released fat droplets which entered the bile canaliculi or blood sinusoids and thus entered the bilary and vascular systems. It was concluded that fat droplets released in this manner could cause embolic lesions in the heart. On the other hand, studies by Wilgram, Best and Blumenstein ('55) revealed that female and older rats were less sensitive to the ill effects of choline deficiency and rarely exhibited cardiovascular lesions. Lesions did not appear in these animals which were less sensitive to acute cardiovascular experiments although it was possible to produce coronary and aortic changes by prolonged deficiency of choline. These investigators agreed with the hypothesis that the cardiovascular lesions induced by acute choline deficiency were linked to kidney damage. A diseased kidney might conceivable be unable to regulate phosphorous and calcium levels in plasma and tissues and the resulting disturbance would favor the production of vascular damage. On the other hand rats have a greater ability to form phosphatidyl choline than the chick which may be one

reason why rats are rather resistant to induced arteriosclerosis.

It appears that the length of the experimental period may be a factor which prevent the occurrence of any structural changes in the hearts of mice maintained on a low protein diet. Since cardiovascular lesions did not occur in hearts of the mice fed on this diet, it may be that the length of the experimental period was not sufficient to allow for physiological or biochemical changes involved in their production.

CHAPTER VI

SUMMARY AND CONCLUSIONS

1. Mice were fed a low protein diet over a period of 10 days in order to determine whether or not such a diet would produce any gross and/or histological changes in their hearts.

2. There were no structural changes observed in mice fed the low protein diet over a period of 10 days.

3. Mice maintained on the experimental diet lost body weight.

4. The short length of the experimental period may account for the lack of structural changes in the hearts of the experimental mice. Biochemical and physiological changes involved in the production of cardiovascular lesions did not develop within this period.

5. It is suggested that the low protein diet may have affected the hearts if the investigation had been extended over a longer period of time.

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TABLE 1

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WEIGHT CHANGES AND THE AMOUNTS OF FOOD INGESTED BY ALBINO MICE MAINTAINED ON THE CONTROL AND LOW PROTEIN DIETS

	CONTROLS				EXPERIMENTALS			
Days	Initial Weight	Weight when Sacrificed	Food Ingested	Weight Gained	Initial Weight	Weight when Sacrificed	Food Ingested	Weight Lost
1	14.8	15.7	16.2	0.9	16.4	14.8	0.6	1.6
2	15.2	17.0	1.8	1.8	16.6	13.8	9.6	2.8
3	14.8	15.1	1.8	0.3	15.2	11.95	0.5	3.2
4	12.3	12.8	1.4	0.5	15.4	11.3	1.7	4.1
5	19.5	22.2	2.4	2.7	14.5	9.7	1.4	4.8
6	16.0	16.3	2.5	0.3	14.8	10.9	2.4	3.9
7	17.8	20.8	1.6	3.0	17.0	11.7	1.9	5.3
8	17.4	17.9	2.8	0.5	17.5	11.5	1.6	6.0
9	13.5	15.8	3.8	2.3	17.5	12.2	0.5	5.3
10	16.4	26.6	3.2	10.2	18.5	11.2	1.3	7.3

*

All data is expressed in grams.

PLATE I

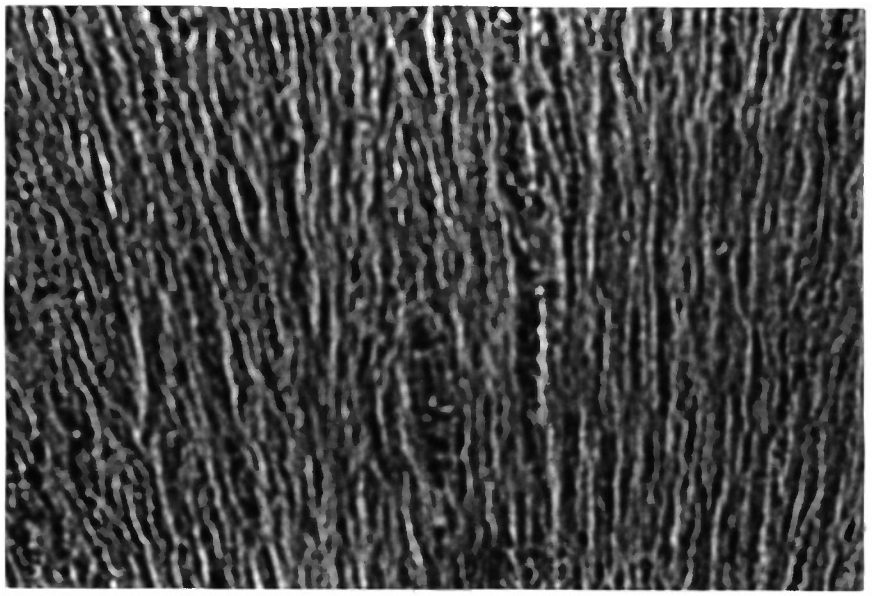
(Explanation of Figures)*

*
All figures are photomicrographs.

(Explanation of Figures)

Fig. 1. Section from the heart of a control mouse showing muscle tissue of the ventricle. X 430.

Fig. 2. Section from a heart of a control mouse showing the epicardial region covered on its free surface by a single layer of mesothelial cells. X 430.



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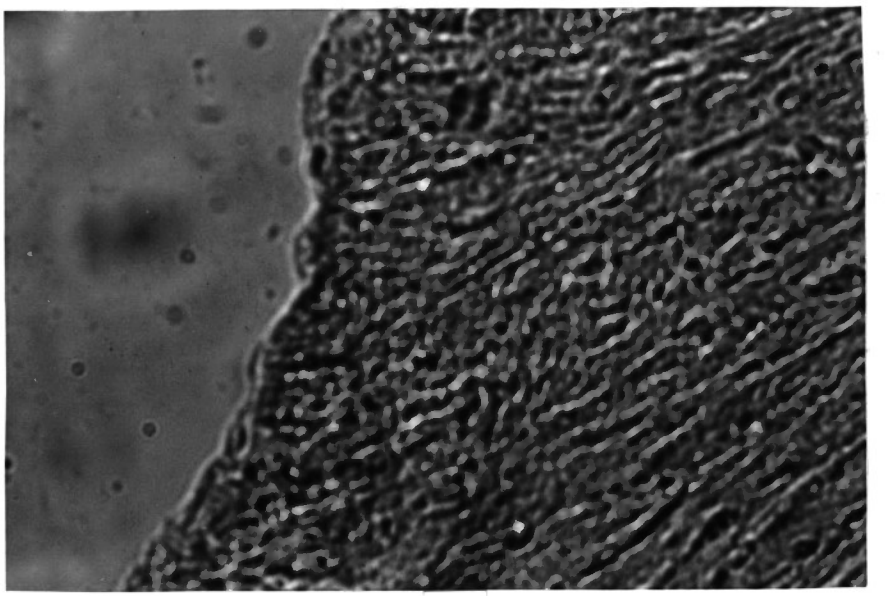

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PLATE II

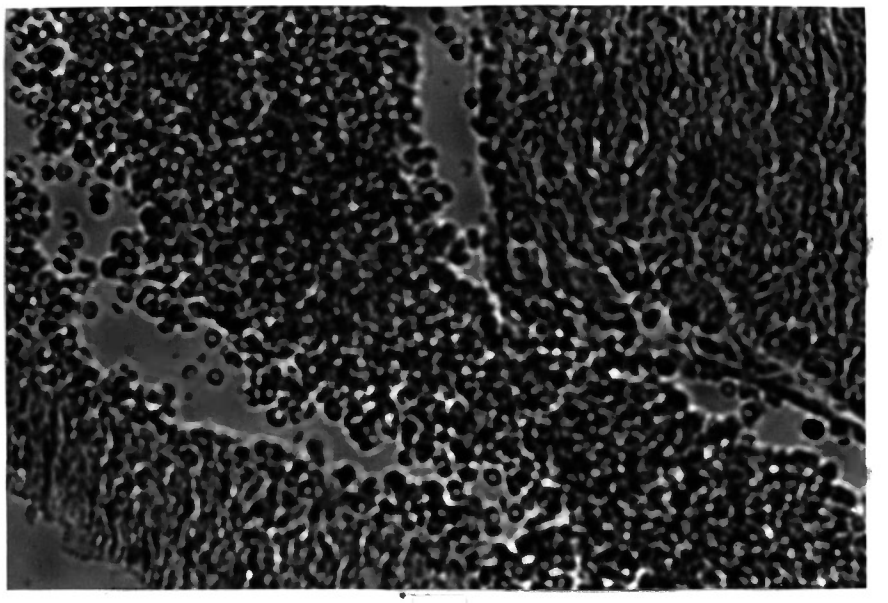
(Explanation of Figures)*

* All figures are photomicrographs.

(Explanation of Figures)

Fig. 3. Section of a heart of a control showing portion of a vein. X 430.

Fig. 4. Section of a heart of a 10-day experimental mouse showing an artery, muscle tissue and the mesothelial layer of cells. X 430.



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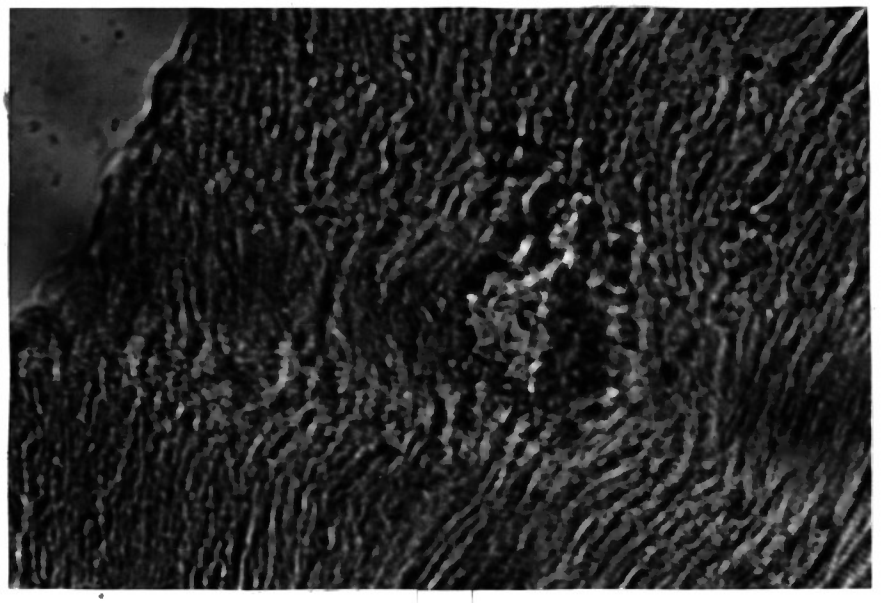

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PLATE III

(Explanation of Figures)*

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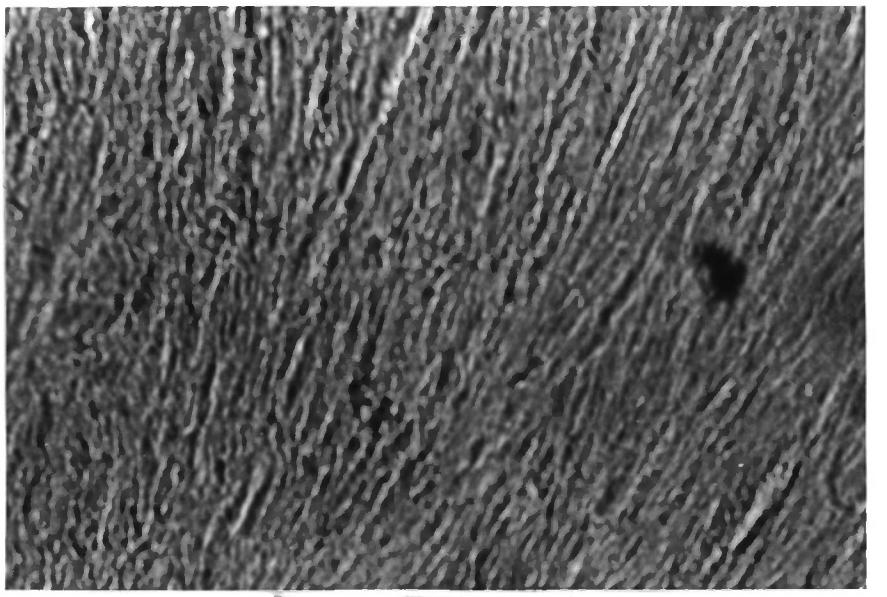
All figures are photomicrographs.

(Explanation of Figures)

Fig. 5. Section of a heart of a 7-day experimental showing cardiac ventricular muscle. X 430.

Fig. 6. Section of a heart of a 9-day experimental showing an artery. X 430.



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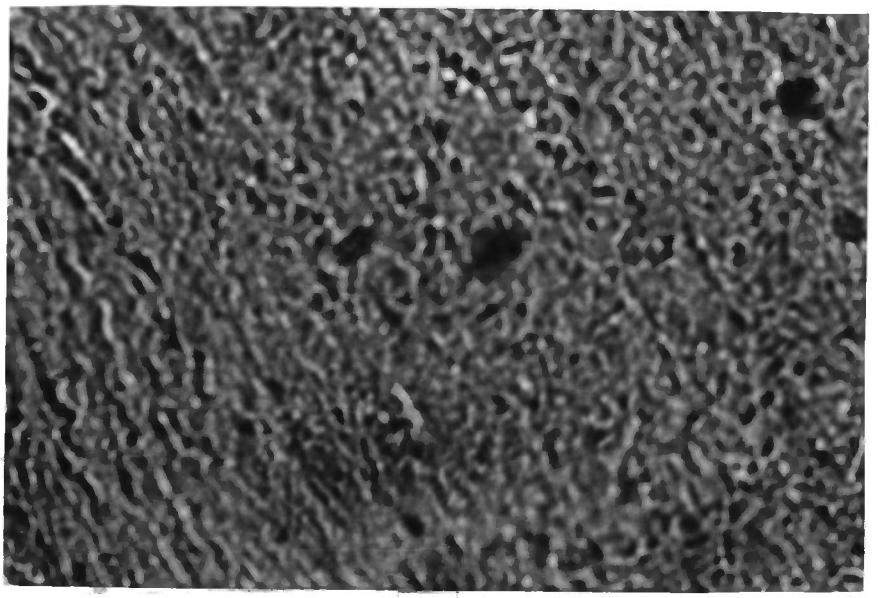

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PLATE IV

(Explanation of Figures)*

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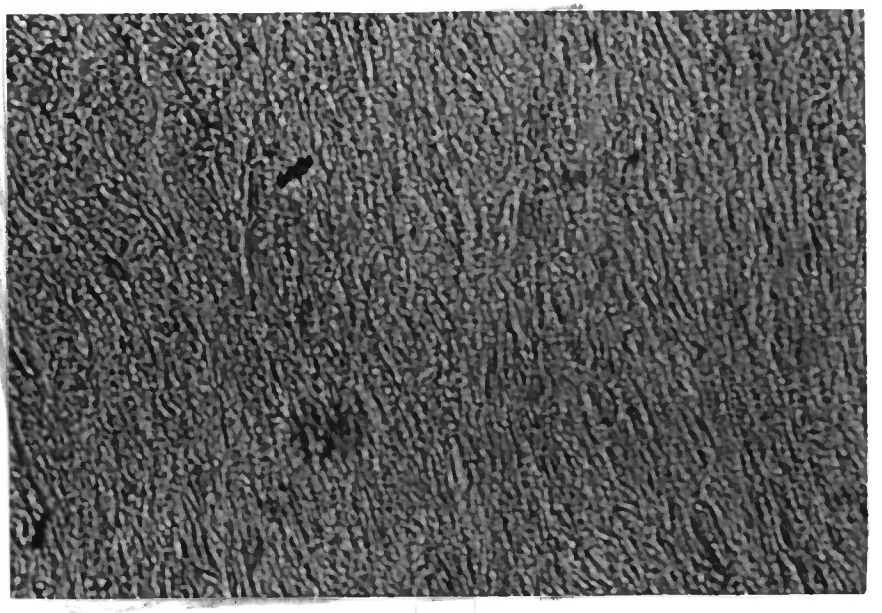
All figures are photomicrographs.

(Explanation of Figures)

Fig. 7. Section of a heart of a 4-day experimental showing the endocardium of the ventricle. X 430.

Fig. 8. Section of a heart of a second day experimental showing a vein. X 430.



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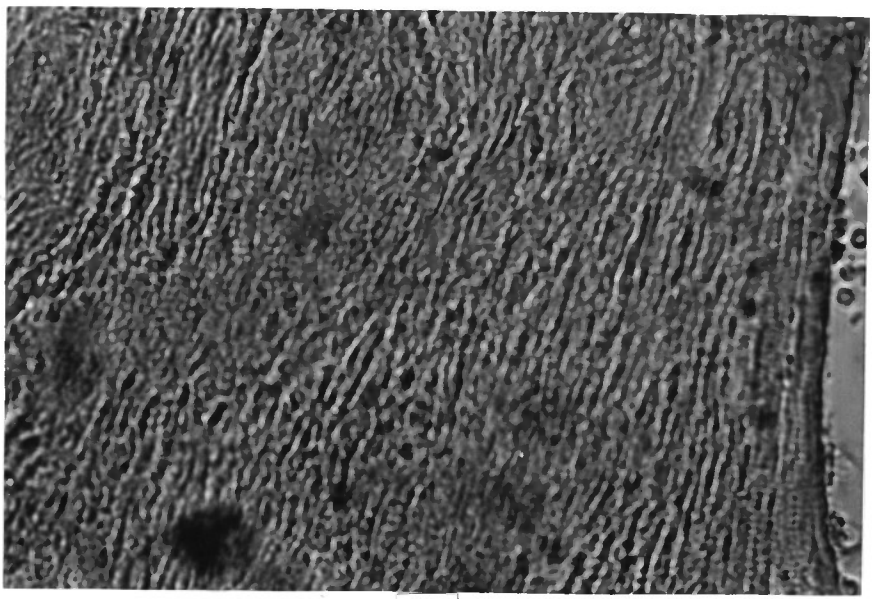

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PLATE V

(Explanation of Figures)*

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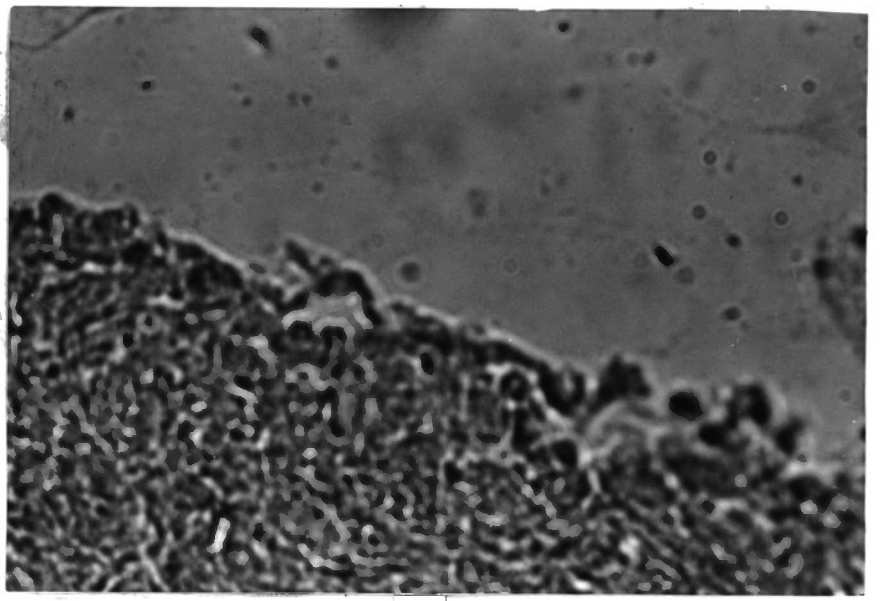
All figures are photomicrographs.

(Explanation of Figures)

Fig. 9. Section of a heart of a 6-day experimental showing muscle tissue covered on its free surface by a single layer of mesothelial cells. X 430.

Fig. 10. Section of a heart of an 8-day experimental showing muscle tissue, blood vessel, and a layer of mesothelial cells. X 430.



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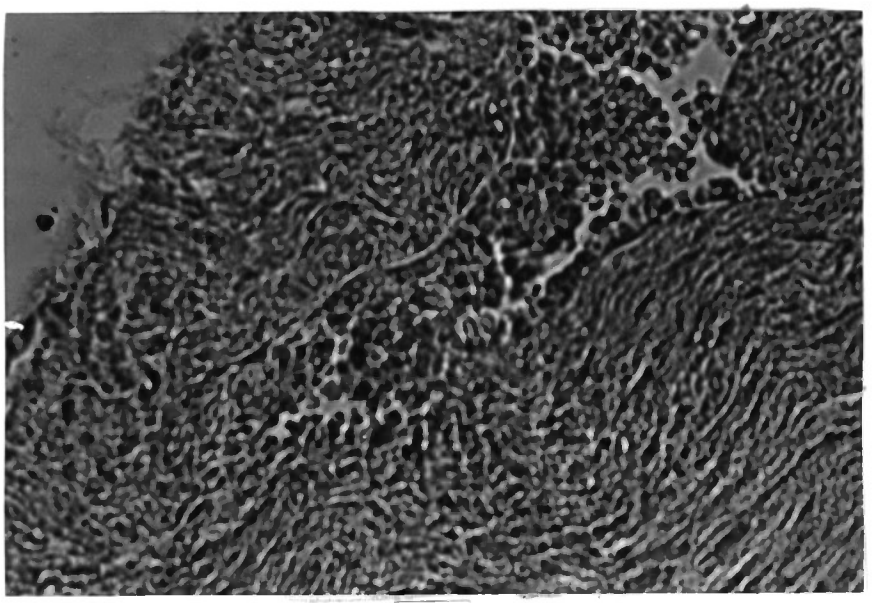

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